BIOCHEMICAL GENETICS OF NEUROSPORA

E. L. TATUM AND G. W. BEADLE

Stanford University, California

The production by irradiation of mutant strains of *Neurospora* (Beadle and Tatum, '41) has provided material for the cooperative attack by genetical and biochemical methods on the problem of the mechanism of gene action (Tatum, '44, Horowitz *et al.*, '45, Beadle, '45a). The genetic approach has shown that of the mutant strains so far investigated, both the morphological and biochemical ones are differentiated from normal by single genes. Genetic methods based on crosses and on the formation of heterocaryons have been developed for establishing the allelic or non-allelic nature of specific genes, and for determining the relative dominance of particular biochemical genes (Beadle and Coonradt, '44). The genetic analysis of mutant strains should in time provide data for the location of the mutant genes on chromosome maps. Already quite a few genes have been so located on one or another of the seven chromosomes of *Neurospora* recently demonstrated by McClintock (unpublished).

The biochemical investigations have supported the view that genes controlling biosyntheses of vitamins and amino acids and other biologically important substances in *Neurospora* act through their primary effect in determining the specificity or the production of enzymes involved in carrying on individual steps in the biosyntheses. The mutant strain is perhaps characterized by total or partial failure of enzyme synthesis. Another possibility would be the production of a modified enzyme with altered specificities, which is as a result either inactive or less effective than the normal enzyme in catalyzing the required reaction. If the general concept of a biosynthesis as a sequential series of enzymic reactions is correct, a number of consequences may be predicted which can be tested experimentally. If a given gene affects only one enzyme and therefore only one biochemical reaction, each enzymic step should be controlled by a different gene. Or conversely, two non-allelic genes controlling even the same synthesis must affect different biochemical reactions in that synthesis. It should also follow that an intermediate compound preceding a genetically blocked reaction should be inactive, while one following this point in the biosynthesis might be expected to show the same order of activity as the end product.

The results of investigations have so far supported these predictions. At least seven different genes are known to be involved in the synthesis of arginine. (Srb and Horowitz, '44). Of these, four are concerned in unknown reactions leading to the synthesis of ornithine, two in the conversion of ornithine to citrulline, in which two biochemical steps have been suggested (Krebs, '36), and only one in the conversion of citrulline to arginine, a reaction involving only one obvious step. At least two genes are concerned in the synthesis of tryptophane, one in the production of anthranilic acid and one in the conversion of anthranilic acid

to indole (Tatum, Bonner and Beadle, '44). The latter is then converted to tryptophane by condensation with serine (Tatum and Bonner, '44), a reaction for which no mutant gene has yet been found in *Neurospora*. In the synthesis of thiamin one gene governs the synthesis of thiazole, and another the condensation of thiazole with pyrimidine (Tatum and Bell, unpublished). Another gene is known which apparently controls the synthesis of pantothenic acid from its components, β-alanine and pantoyl-lactone, either one or both of which are inactive for the mutant strain.

A third possible consequence of the original assumptions would be the accumulation of an intermediate the further conversion of which is blocked by the gene mutation. A few examples of this accumulation of intermediate products in *Neurospora* mutant strains are known. The *indoleless* strain 10575 which can form tryptophane from indole but not from anthranilic acid actually produces this latter compound, which must therefore be an intermediate in tryptophane synthesis in *Neurospora* (Tatum, Bonner and Beadle, '44). *Thiazoleless* 18558 produces vitamin pyrimidine, while *thiaminless* 9185 produces both thiamin intermediates but cannot bring about their coupling (Tatum and Bell, unpublished). *Pantothenicless* 5531, which requires intact pantothenic acid, synthesizes both β-alanine and pantoyl-lactone (Tatum, unpublished). Not only has the accumulation of intermediates of known constitution been shown to result from particular gene mutations, but also the production of intermediates of unknown nature. The isolation and identification of these should give some insight into as yet unknown biochemical mechanisms of certain other syntheses. Such an intermediate is produced by *cholineless* 47904 but has choline activity for strain 34486 in which the biosynthesis is blocked at an earlier step (Horowitz, unpublished). Another instance is the production of a nicotinic acid precursor by strain 4540 which is capable of replacing this vitamin for another mutant strain (39401) (Bonner, unpublished). This nicotinic acid precursor instead of accumulating is, under some conditions, further metabolized, apparently with the production of an inactive, intensely colored yellow pigment. An inactive purple-colored compound is apparently formed as the result of a reaction involving an intermediate in the synthesis of adenine (Mitchell, unpublished).

Unfortunately, the accumulation of active intermediates in mutants of *Neurospora* seems to be the exception rather than the rule. In many cases this seems to be due to their lability, which results in the further metabolizing of these products as rapidly as they are formed. Other difficulties in the isolation and identification of these substances are the small amounts produced and the narrow range of cultural conditions under which they can be shown to accumulate. Nevertheless, the results of these investigations of mutant strains suggest that in each a single reaction is blocked, and are consistent with the hypothesis of a one-to-one relation between gene and chemical reaction, through specific enzymes.

A few mutants have been found in which more complex reactions or require-

ments have been indicated. Cases in which the activity of the single essential substance is increased by the addition of a second substance can be interpreted as secondary effects, due to biochemical relations not necessarily connected with the blocked biosynthesis. Examples of this are the sparing action of methionine on *cholineless* (Horowitz and Beadle, '43) and possibly that of thiamin on *pyridoxinless* (Stokes, Foster and Woodward, '43). One well-established actual double requirement resulting from a single gene mutation is that of the two amino acids, isoleucine and valine. The close biochemical relation of these two makes plausible the assumption that their biosyntheses involve either a common precursor or a common enzymatic reaction (Bonner, Tatum and Beadle, '43). This interpretation is consistent with the hypothesis that a one-to-one relation exists between the gene and a given enzyme and primary reaction.

Another instance of a double requirement is known, the basis of which is not so easily interpreted. In strains 17084 and 1090 single-gene mutations apparently block the synthesis of both thiamin intermediates, thiazole and pyrimidine (Tatum and Bell, unpublished). Since there is no obvious biochemical similarity in these compounds the interpretation of the action of the genes concerned on the basis of a single reaction is difficult. One possible interpretation is that the synthesis of only one component is blocked, and that the inactive intermediate which is formed then combines or reacts with the other component or its precursor, thus resulting in an actual deficiency in both. An exogenous supply of both compounds is apparently completely active for these mutant strains. The results of further study of these strains will be of the utmost importance in connection with the general validity of the proposed one-to-one relation of gene and enzyme.

Two fairly common and possibly related phenomena have been met with in *Neurospora* as well as in other micro-organisms. These are cases in which a requirement for a specific substance is altered or dispensed with, the result of prolonged incubation in deficient media, "adaptation" (Bonner, Tatum and Beadle, '43), or as an immediate response to altered cultural conditions. In general, it has been found in *Neurospora* that in both instances the genetic constitution of the modified strains is unaltered. There are two possible explanations for these phenomena. One, suggested for *pyridoxinless* by Stokes *et al.* ('43), is that the gene mutation has resulted in a limitation of the physiological conditions under which the synthesis can be performed by a given mutant strain. This could imply the production by the mutant of an enzyme with more restricted capacities. The other possibility is that there may be alternative mechanisms for carrying on certain syntheses or certain steps in a synthesis, and that these different mechanisms may normally function under different physiological conditions. In this case the gene mutation has resulted in the failure of only one synthetic mechanism. The search for mutants of this type has led to the discovery of a number which require particular substances only under definite conditions, especially at temperatures over 28° C. The substances required by these "temperature" mutants include riboflavin, adenine and uracil (Houlahan and Mitchell, unpublished).

It is possible that the phenomenon of adaptation is essentially analogous to alterations in synthetic capacities in response to external environmental changes. Adaptation could result from either (1) the formation of an adaptive enzyme capable of carrying on an alternative synthetic reaction, the enzyme possibly formed in response to the slow accumulation of the intermediates (substrate), or (2) the lag in adaptation could be due instead to a slow modification in the internal cellular environment, a change eventually leading to the functioning of an alternative reaction, or of the original reaction under the modified conditions in the cells. Further experimental evidence may permit a decision as to whether or not these phenomena involve different reactions. The results should have a direct bearing on the validity of the one-to-one relation of gene and reaction.

The pleiotropic manifestations of certain genes in other organisms may at first glance seem difficult to reconcile with the one-to-one gene to enzyme concept. A possible solution is that relating such effects to multiple functions of the primary gene product (Beadle, '45b). It is possible that certain instances in *Neurospora* may be of value in analyzing some effects of this nature at the biochemical level. In a number of biochemical mutant strains, the primary deficiency is accompanied by a specific sensitivity to other compounds, lacking in other mutant strains and in the wild type. The best analyzed and most striking instance in *Neurospora* is the specific inhibition of *lysineless* by arginine (Doermann, '44). This action of arginine has been interpreted as an inhibition of the utilization of the required lysine by the arginine supplied. The utilization of endogenous lysine in the wild type is not interfered with by exogenous arginine. The different effect of arginine in the two cases may be due to differences in the metabolism of exogenous and endogenous lysine. Other instances also suggest the existence of differences between the physiological action and therefore of the metabolism of exogenous and endogenous materials of biological importance. For *tryptophaneless* mutant strain 10575 indole has a greater molar activity than has tryptophane. This apparently results from the more rapid destruction of added tryptophane than of the tryptophane formed *in situ* from indole. Thiazole has a three- to four-fold greater antipyrithiamin activity than does thiamin (Tatum, unpublished). If pyrithiamin inhibits the utilization of thiamin as suggested by Woolley and White ('43) and by Sarett and Cheldelin ('44), thiamin synthesized in the cell from thiazole must be more effective in antagonizing pyrithiamin than is exogenous thiamin. Houlahan and Mitchell (unpublished) have found with a mutant strain which requires riboflavin at higher temperatures that the growth response to riboflavin is very strongly inhibited by lumichrome, and somewhat by lumiflavin, neither of which inhibits the growth of other strains. These results again suggest differences in the metabolism of endogenous and exogenous substances. The many examples of amino acid inhibitions and antagonisms reported with bacteria and the specific inhibitions noted in *Neurospora* mutant strains, all indicate the complexity of interactions of substances in the living cell, and suggest some of the difficulties to be met with in interpreting data

on growth requirements. These interrelations and interactions may arise in part from differences in the biochemical fates of substances of endogenous and exogenous origin, and may provide a biochemical basis for the explanation of certain types of multiple gene effects.

In conclusion, the results so far obtained with *Neurospora* support the hypothesis that genes concerned in biosyntheses, and probably all genes, act in a primary way by determining the specificity of, or in controlling the production of enzymes. The results also support the view that a one-to-one relation exists between gene and enzyme. At present it seems likely that any apparently multiple gene effects in *Neurospora,* when completely analyzed biochemically and genetically, will be found to be due to common primary reactions, or to secondary interactions not directly related to the action of the mutant gene under consideration.

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